THE CRYSTAL AND MOLECULAR STRUCTURE OF SOME ORGANIC POLYSULPHIDES

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AN X-RAY INVESTIGATION OF THE EFFECT OF RICKETS ON BONE STRUCTURE IN RATS

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SYNOPSIS

In the investigation of the structure of some organic polysulphides an account is given of unit cell and space group measurements for diphenyl disulphide, di-p-tolyl disulphide, and β,β'-diiododiethyl trisulphide. Preliminary structure analysis by the method of Patterson synthesis is reported in the case of di-p-tolyl disulphide, followed by the results of the complete analysis of the β,β'-diiododiethyl trisulphide structure. A chain-like structure has been established for the molecule $I.CH_2.CH_2.S.S.S.CH_2.CH_2.I$. A sulphur-sulphur bond length of 2.04 ± .04 A and an S - S - S bond angle of 113° ± 2°, have been obtained from a two-dimensional Fourier projection.

In the additional paper the results are given of a comparative study of the structure of rachitic and of normal bone by X-ray diffraction methods. It has been found that, in the rachitic rat, no visible change in the diffraction pattern occurs. The impaired mechanical properties of rachitic bone are related to changes in the proportion of inorganic material laid down rather than to change in the structure of the inorganic salt itself.

INTRODUCTION

a) Historical

From observations made on calcite crystals Hauy (1784) recognised that the regularity of crystalline form was due to an underlying regular structure. The science of classical crystallography thus developed, involving in its chemical aspects the correlation of crystal habit with chemical constitution and in its geometrical aspects a study of the symmetry combinations possible for space lattices. In the field of chemical crystallography Groth's[1.] treatise summarises and tabulates the data available up to 1905. The geometrical problems were first investigated by Frankenheim and Bravais. The 230 space groups denoting the number of ways in which asymmetric units could be arranged in space were finally formulated independently by Federov (1890), Schoenflies (1891) and Barlow (1894).

With Laue's[2.] discovery of X-ray diffraction in 1912 direct evidence was obtained of the wave nature of X-rays. It was quickly recognised that a method was now available for the interpretation of crystal structure in terms of atomic and molecular dimensions and in 1913 W.L. Bragg[3.] published his results on the analysis of sodium chloride and potassium chloride. W.H. Bragg's[4.] development of the ionisation spectrometer provided a useful experimental method, which was employed in most of the early structural investigations.

In 1915 W.H. Bragg[5.] developed the idea of the application of

Fourier series to the problems of X-ray analysis. He emphasized that the coefficients of the Fourier series which represents the periodic variation of density in the medium should be proportional to the intensities of the corresponding X-ray reflections. Prior to this in 1914 Darwin[6] had given a complete theoretical treatment of the intensity of the X-ray reflection in which the formulae for the intensity from both perfect and mosaic crystals were derived. Compton[7] deduced the relationship between observed intensities and the coefficients of the Fourier series and the method was first employed by W.L. Bragg[8] in 1929 in a study of the structure of diopside.

During this time corresponding developments had taken place on the experimental side. Powder methods had been introduced by Debye and Scherrer[9] and Hull[10] thus extending the scope of the X-ray method to microcrystalline materials. The rotating crystal method was developed as a result of the work of Schiebold and others and the original Laue method was improved by Wyckoff[11], Pauling[12] and Schiebold[13]. In 1924 Weissenberg[14] introduced the moving film method of recording X-ray reflections photographically. This, in conjunction with the concept of the reciprocal lattice introduced by Ewald[15] made space group determination a routine matter and by decreasing the intensity of incoherent scattering relative to that given by coherent scattering considerably increased the accuracy possible in intensity measurements by photographic methods.

The complexity of most of the organic molecules which are crystalline at ordinary temperatures made the elucidation of their structure extremely difficult, and it was not until 1929 that the first of these, hexamethylbenzene, was determined in full by Lonsdale.[16] The first Fourier analysis of a complex organic molecule was carried out on anthracene by Robertson.[17] In the succeeding decade numerous other organic structures were successfully examined.

In the early work it was necessary to proceed by trial and error methods. A probable structure for the molecule was postulated and from it the intensities of the X-ray reflections calculated. When a sufficiently good agreement was obtained between calculated and observed intensities the structure was then refined by successive Fourier syntheses. This process was necessarily a laborious one. Considerable advance was made possible by the use of heavy atom method developed by Robertson[18] in work on the compounds of the phthalocyanine series, and the introduction of the direct Fourier method by Patterson.[19] By a combination of these two techniques it is possible to examine crystals about which no information regarding probable atomic arrangement is available.

b) Theoretical

Diffraction by the Crystal Lattice

In a true crystal the unit of pattern is repeated in three dimensions and this periodicity in the crystalline structure acts as a diffraction grating for X-rays. The effect is analogous to that of the diffraction of light by a line grating. When light is diffracted by a line grating the positions of the spectra depend only on the wavelength of the light employed and the grating constant, i.e. the interval at which the lines repeat. Similarly in the case of X-ray diffraction by crystals the positions at which the spectra appear are dependent on the intervals at which the pattern repeats in three dimensions. The conditions for the formation of X-ray spectra were first formulated by Laue. If the incident beam makes an angle ψ_0 with the grating, the spacing of which is $\underline{a}$, and the diffracted beam makes an angle ψ with the grating, then the path difference is $a(\cos\psi - \cos\psi_0)$ or $a(\alpha - \alpha_0)$ where $\cos\psi = \alpha$ and $\cos\psi_0 = \alpha_0$. Reinforcement occurs when the path difference is equal to an integral number of wave lengths. $n\lambda$ where λ is the wave length and $\underline{n}$ an

i.e. $a(\alpha - \alpha_0) = n\lambda$ where λ is the wave length and $\underline{n}$ an integer.

For the three dimensional case we must have three such relations, viz:

$$\left.\begin{aligned} a(\alpha - \alpha_0) &= h\lambda \\ b(\beta - \beta_0) &= k\lambda \\ c(\gamma - \gamma_0) &= \underline{l}\lambda \end{aligned}\right\} \quad \ldots\ldots\ldots\ldots\ldots\ldots(1)$$

The order of the resultant spectrum being defined by the three integers h, k, and l.

Bragg considerably simplified the theoretical treatment by considering the phenomenon as a reflection from successive planes of the crystal lattice. Fig. 1 illustrates this concept. The incident X-ray beam impinges on a series of planes whose spacing is d.

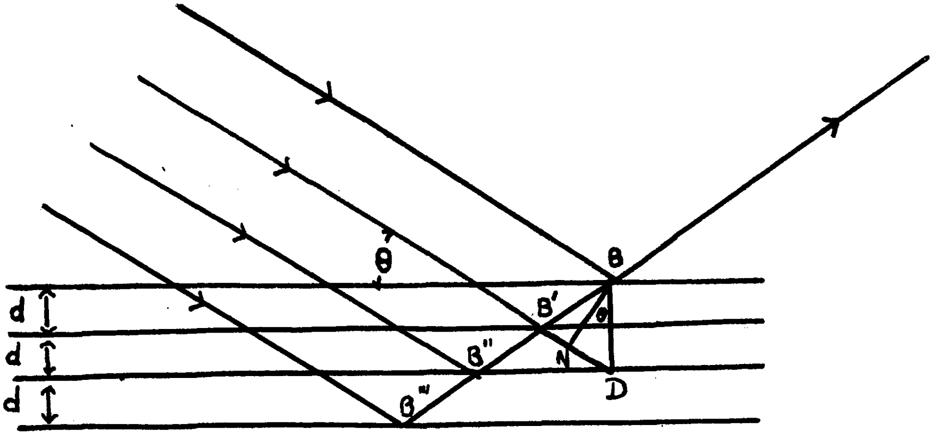

Fig. 1

When the angle of incidence is θ the path difference between reflections from successive planes is $B^1B - B^1N = B^1D - B^1N = DN = 2d\sin\theta$. With monochromatic radiation λ is fixed in the relation

$$n\lambda = 2d\sin\theta \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

which is the condition for reinforcement from successive planes. Hence enhancement will only occur for certain definite values of $\underline{\theta}$ corresponding to integral values of $\underline{n}$. On the other hand if we employ a 'white' X-radiation $\underline{\lambda}$ is a variable in the above relationship $n\lambda = 2d\sin\theta$ and for a fixed value of $\underline{\theta}$ several reinforcements will occur .

For a given wavelength only a finite number of spectra can occur the limit being reached as $\sin\theta$ approximates to unity.

Determination of unit cell dimensions

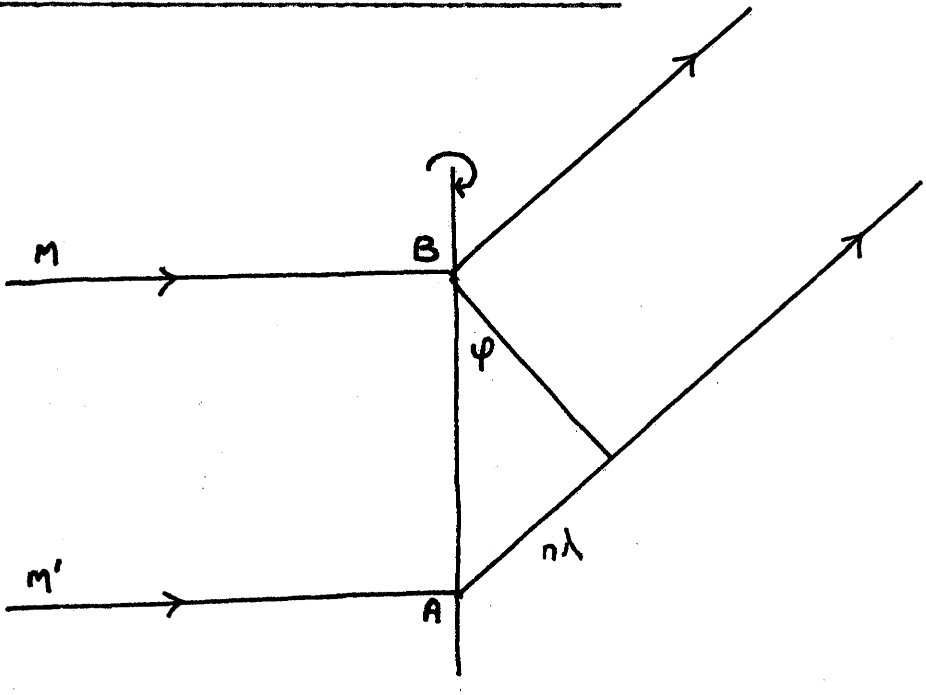

Fig. 2

Let a crystal be rotated about a certain axis with an X-ray beam MM^1 impinging on the crystal normal to that axis. Then if

A and B are successive identical points along the axis of rotation the direction of diffraction will depend on AB according to the relation $n\lambda = AB \sin\varphi$ as is seen in Fig. 2. φ is the complement of the angle between the diffracted beam and the axis of rotation. If BA represents the a axis of the crystal all a reflections for which $h = n$ have directions of diffraction such that they make the same angle $(90^\circ - \varphi)$ with the axis of rotation. Hence, all (1 k 1) reflections will lie on a cone with axis, the axis of rotation, all (2 k 1) reflections will lie on a concentric cone with a smaller semi-vertical angle. If a flat plate is used to record the reflections the successive cones will intersect the plate in hyperbolas, while if a cylindrical film is placed with the axis of rotation coinciding with the axis of the cylinder then the cones will intersect the film in circular paths, the so-called layer lines of the rotation photograph. The distance between the layer lines can be measured and from the radius of the camera the angle φ is obtained for each layer line. The periodicity along the axis can then be determined from the relation $n\lambda = a \sin\varphi$ where n is the order of the line above the equatorial line and φ the corresponding diffraction angle. Hence periodicities can be measured in three directions and the unit cell determined.

The Structure Amplitude $|F(hkl)|$

Examination of a crystal by X-ray methods gives information on the position and intensity of the recorded reflections. The structure amplitude $|F(hk\underline{l})|$ is defined as the ratio of the amplitude of the reflected ray actually received for the plane $(hk\underline{l})$ to that which would be received if the contents of the cell were replaced by a single electron. The diffracted wave is made up from components from each atom in the unit cell, hence if we consider an atom of coordinates (x, y, z) then the difference in phase of the wave scattered by this atom compared with that at the origin will be $2\pi(hx/a + ky/b + \underline{l}z/c)$ where $\underline{a}$, $\underline{b}$ and $\underline{c}$ are the axial lengths of the unit cell. As the structure amplitude in the general case includes both amplitude and phase it can best be expressed by the use of complex numbers.

$$|F(hk\underline{l})| = \sum f e^{2\pi i(hx/a + ky/b + \underline{l}z/c)} \qquad \ldots\ldots\ldots\ldots(3)$$

the summation being carried out over all atoms in the unit cell. $\underline{f}$ is the scattering efficiency of each atom for the particular plane considered so that $|F(hk\underline{l})|$ is given in absolute units in accordance with the definition given above. $|F(hk\underline{l})|$ must be on such a scale that its value when $\sin\theta = 0$ is equal to the number of electrons in the unit cell. $\underline{f}$ falls away rapidly with increase of $\frac{\sin\theta}{\lambda}$ as the efficiency of scattering is reduced for large values of $\underline{\theta}$ by phase differences between the component waves scattered by electrons in the different parts of the atoms. The scattering of X-rays by an atom of an element depends on the atomic number and $\underline{f}$ may be calculated from the expression

$$f = \int_0^\infty U(r)\,\frac{\sin\varphi}{\varphi}\,dr \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots(4)$$

where $\varphi = 4\pi r \frac{\sin\theta}{\lambda}$ and represents the probability of finding an electron between the radius r and r + dr from the centre of the atom.

Space Group Theory

A crystal regarded as a whole possesses certain elements of symmetry and if grown under ideal conditions this symmetry will be apparent in the external form of the crystal. This symmetry is also indicated by the physical properties of the crystal, e.g. thermal expansion, electrical conductivity and optical properties. The operations necessary to describe completely the external symmetry of a crystal are those of reflection, rotation and rotation combined with inversion.

A crystal has in general a number of these symmetry elements forming an associated group which describe its symmetry; the combination is termed a point group. In all we find that there are 32 different combinations, distributed among the seven crystal systems, triclinic, monoclinic, orthorhombic, hexagonal, rhombohedral, tetragonal and cubic. To extend the theory to a continuous lattice it is necessary to consider two additional elements of symmetry. These are the glide plane and screw axis, both of which involve translational movements.

This problem was examined independently by Schoenflies, Federov and Barlow. They concluded that there are 230 geometrically distinct ways of arranging the symmetry elements possible in a crystal. Every crystal found in nature must conform to one of these groups, although every group may not be physically possible.

As has been shown in equation (3) the structure amplitude for any plane $(hk\underline{l})$ is given by the expression

$$|F(hk\underline{l})| = \sum f e^{2\pi i (hx/a + ky/b + \underline{l}z/c)}$$

where the complex resultant expresses both the amplitude and phase of $|F(hk\underline{l})|$ according to the usual conventions.

This equation (3) involves sine terms as well as cosine terms but if the structure has a centre of symmetry then an atom in position (x, y, z) will have a related atom at $(\bar{x}, \bar{y}, \bar{z})$ and for each term in the summation $\sin 2\pi(h\bar{x}/a + k\bar{y}/b + \underline{l}\bar{z}/c)$ there will be a term $\sin 2\pi(hx/a + ky/b + \underline{l}z/c) = - \sin 2\pi(h\bar{x}/a + k\bar{y}/b + \underline{l}\bar{z}/c)$. Hence the sine terms will cancel out and in this special case

$$F(hk\underline{l}) = \sum f \cos 2\pi(hx/a + ky/b + \underline{l}z/c) \quad \ldots\ldots\ldots(5)$$

Now, for example, if we consider a structure composed of atoms, on a face-centred lattice with an origin at one of the atoms, other atoms in the lattice are at the points $0\tfrac{1}{2}\tfrac{1}{2}$, $\tfrac{1}{2}0\tfrac{1}{2}$, $\tfrac{1}{2}\tfrac{1}{2}0$. The structure factor, since the sine terms are zero, is given by the expression

$$\begin{aligned} F(hk\underline{l}) &= 4f\left\{\cos 0 + \cos 2\pi(\tfrac{1}{2}k + \tfrac{1}{2}\underline{l}) + \cos 2\pi(\tfrac{1}{2}h + \tfrac{1}{2}\underline{l}) + \cos 2\pi(\tfrac{1}{2}h + \tfrac{1}{2}k)\right\} \\ &= 4f \cos\pi(h + k + \underline{l})\cos\tfrac{1}{2}\pi(k + \underline{l})\cos\tfrac{1}{2}\pi(h + \underline{l})\cos\tfrac{1}{2}\pi(h + k) \quad \ldots\ldots(6) \end{aligned}$$

This expression covers all the possible reflections from a face-centred lattice and it is seen that the expression vanishes for odd values of $h + l$, $K + l$, $h + k$, i.e. h, k and l, are either all even or all odd integers. The spectra (120) (330) etc. cannot occur and are known as 'missing spectra'.

The determination of the space group of a crystal by X-ray methods makes use of this fact, since the systematic extinctions can be observed and the symmetry elements necessary for these extinctions determined.

Centred lattices give the most general extinctions, face- or body- centred lattices give extinctions through all values of (hkl). Glide planes have a more limited class of missing spectra one zone only being affected, e.g. if we have a glide plane involving reflection across b accompanied with a translation of $\frac{a}{2}$ all spectra with an odd value of h will be absent.

The existence of a screw axis is denoted by the absence of certain orders of reflection at right angles to the axis, e.g. if the b axis is a two fold screw axis then for the ($0k0$) reflections, only the (020), (040), (060) order will occur. Space Group Theory has been developed in a form suitable for use in conjunction with X-ray analysis by Astbury and Yardley[20.] and in the "International Tables for the Determination of Crystal Structure"[21.] where the extinctions appropriate to the various individual space groups are tabulated.

Certain space groups cannot be established unequivocally by X-ray methods. By Friedel's Law, X-rays, in their diffraction effects, add on the appearance of a centre of symmetry where this may be lacking in the crystal. A case in point is that of di-p-tolyl disulphide considered on p.8 Pt.I.In this crystal the X-ray examination gives a series of absent spectra consistent with space groups $P2_1$ or $P2_1/m$, only the latter possessing a centre of symmetry.

In a crystal which has no centre of symmetry the general face (hk$\underline{l}$) has different properties to the face ($\overline{hk\underline{l}}$). This difference is due to the reversal in the order in which the molecules are encountered in proceeding in one direction or the other normal to the face. It is thus necessary to test for the presence or absence of a centre of symmetry by examining for piezo- or pyro- electric effects before the higher space group of the two alternatives is assumed. If such experiments are inconclusive then the space group of lower symmetry must be assumed.

Further, only symmetry elements producing translations can produce missing spectra. Hencethe space groups P2/a and Pa are indistinguishable by X-ray methods alone as no information about the presence of the two-fold rotation axis can be obtained . General extinctions due to centred lattices imply extinctions in specialised classes, e.g. I 222 cannot be distinguished from I $2_1 2_1 2_1$ as the characteristic screw axis extinctions of (h00) with h odd, (0k0) with k odd and (00$\underline{l}$) with $\underline{l}$ odd occur in any case because of the extinction of (hk$\underline{l}$) with h + k + $\underline{l}$ odd.

In equation (3) the structure factor is obtained in a standard form by summing over every atom in the unit cell. In a given space group, however, there are usually a number of 'equivalent points' and if an atom occurs at one of these it is produced at the others by the symmetry operations of the space group. It is thus found convenient to sum the structure factor over the crystallographically independent atoms alone, and for this the trigonometrical form of the structure factor must be modified from that given in (3) to take account of the equivalent points.

For example the space group $P4_12_1$ in which β,β'-diiododiethyl trisulphide crystallises, there are eight such equivalent points, (International Tables for the Determination of Crystal Structure)[31]. The origin has in this case been taken at the intersection of the two-fold rotation axis perpendicular to (110), with the 4_1 screw axis parallel to c.

(1) $x, y, z.$

(2) $\bar{x}, \bar{y}, 1/2 + z.$

(3) $1/2 - y, 1/2 + x, 1/4 + z.$

(4) $1/2 + y, 1/2 - x, 3/4 + z.$

(5) $y, x, \bar{z}.$

(6) $\bar{y}, \bar{x}, 1/2 - z.$

(7) $1/2 - x, 1/2 + y, 1/4 - z.$

(8) $1/2 + x, 1/2 - y, 3/4 - z.$

The structure amplitude $F(hk\underline{l})$ is then obtained from the following relationships:-

$$F(hk\underline{l}) = \left[(\Sigma f_oA)^2 + (\Sigma f_oB)^2 \right]^{\frac{1}{2}}$$

and the phase angle $\alpha(hk\underline{l}) = \tan^{-1}\frac{\Sigma f_oB}{\Sigma f_oA}$

where

$$A = 4\Big\{\cos\pi(h+k)(x+y)\cos\pi\Big[(h-k)(x-y)-\underline{1}/2\Big\}\cos2\pi(\underline{l}z+\underline{1}/4) +$$
$$+\cos\pi(h+k)\cos\pi\Big[(h-k)(x+y)-\underline{1}/2\Big]\cos\pi(h+k)(x-y)\cos2\pi\underline{l}z\Big\}$$
$$B = 4\Big\{\sin\pi(h+k)(x+y)\sin\pi\Big[(h-k)(x-y)-\underline{1}/2\Big]\sin2\pi(\underline{l}z+\underline{1}/4) -$$
$$-\cos\pi(h+k)\sin\pi\Big[(h-k)(x+y)-\underline{1}/2\Big]\sin\pi(h+k)(x-y)\sin2\pi\underline{l}z\Big\} \quad \ldots\ldots\ldots\ldots (7)$$

Now for the (hk0) zone of reflections this reduces to

$$|F(hk0)| = \sum f_oA$$

and a centro-symmetrical projection is obtained down c. In the investigation of the β,β'-diiododiethyl trisulphide structure it was clear that no resolution could be expected in the c axial projections and it was decided that the (h0l) zone should be examined.

By choosing an origin at the point on a two-fold screw axis parallel to b, at position (-a/4, -b/4) with respect to the 4_1 screw axis, the b axial projections have an apparent centre of symmetry. This origin is situated at the point (a/4, 3b/4, c/8) with respect to the origin adopted in the International Tables for the Determination of crystal structure.[21] Fig. 3 shows the arrangement of the equivalent points in the unit cell.

/Fig. 3.

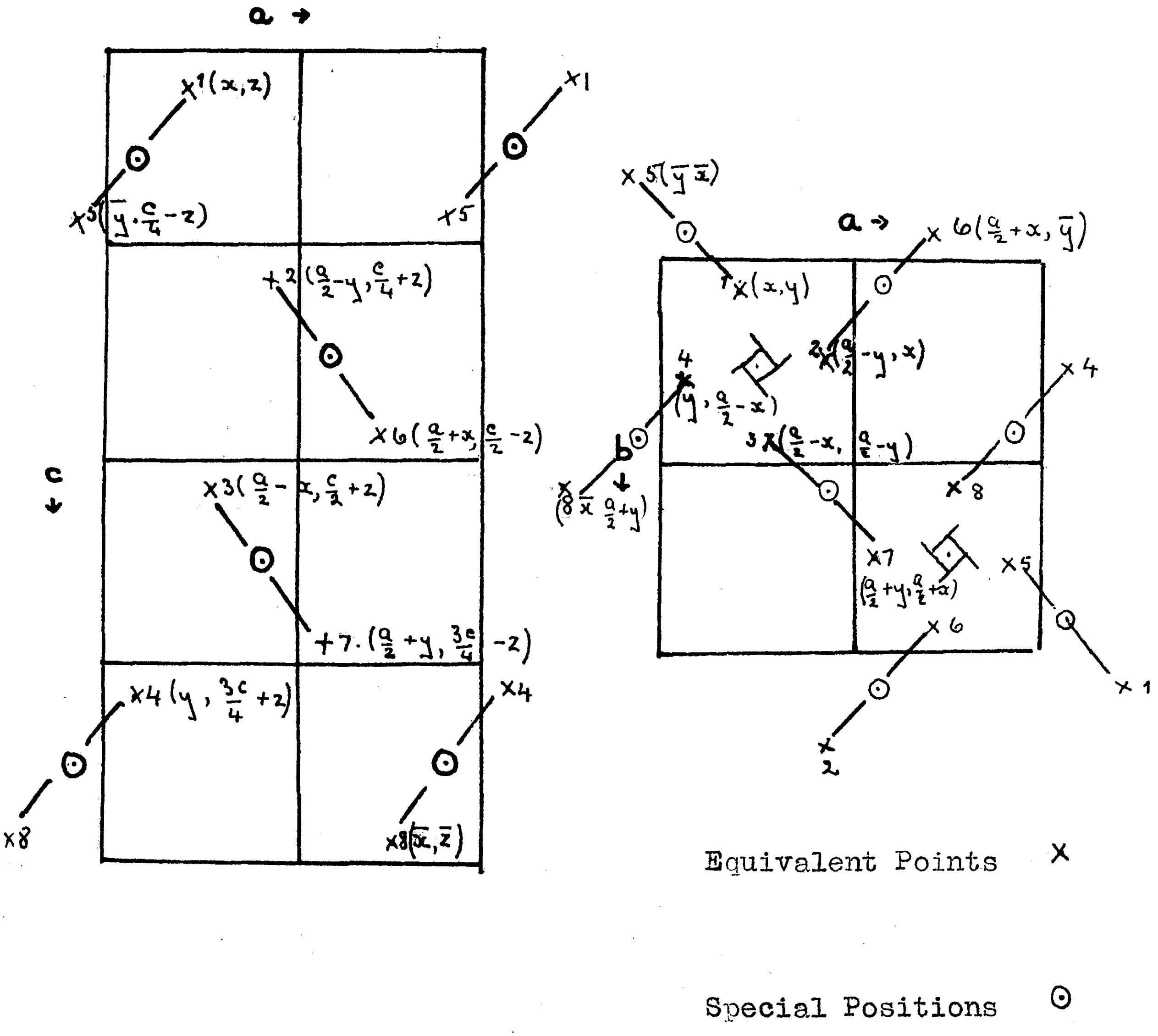

Fig. 3

Arrangement of the equivalent points in space group $P4_1 2_1$.

The coordinates of the equivalent points now become:-

(1) $x,\ y,\ z.$

(2) $\frac{1}{2} - y,\ x,\ \frac{1}{4} + z.$

(3) $\frac{1}{2} - x,\ \frac{1}{2} - y,\ \frac{1}{2} + z.$

(4) $y,\ \frac{1}{2} - x,\ \frac{3}{4} + z.$

(5) $\bar{y},\ \bar{x},\ \frac{1}{4} - z.$

(6) $\frac{1}{2} + x,\ \bar{y},\ \frac{1}{2} - z.$

(7) $\frac{1}{2} + y,\ \frac{1}{2} + x,\ \frac{3}{4} - z.$

(8) $\bar{x},\ \frac{1}{2} + y,\ \bar{z}.$

and the four special positions are at:-

(1) $x,\ \bar{x},\ \frac{1}{8}.$

(2) $\frac{1}{2} + x,\ x,\ \frac{3}{8}.$

(3) $\frac{1}{2} - x,\ \frac{1}{2} + x,\ \frac{5}{8}.$

(4) $\bar{x},\ \frac{1}{2} - x,\ \frac{7}{8}.$

The geometrical structure factor S is then given by

$$S = \sum e^{2\pi i(hx/a \,+\, ky/b \,+\, \underline{l}z/c)}$$

and for $(h0\underline{l})$ i.e. $k = 0$

$$S = \sum e^{2\pi i(hx/a \,+\, \underline{l}z/c)}$$

$$= A + iB \quad \text{and } B = 0$$

since the projection is centrosymmetrical

$$\begin{aligned}
A = {} & \cos 2\pi(hx/a + \underline{l}z/c) + \cos 2\pi\left[h/a(a/2 - y) + \underline{l}/c(c/4 + z)\right] \\
& + \cos 2\pi\left[h/a(a/2 - x) + \underline{l}/c(c/2 + z)\right] + \cos 2\pi\left[\frac{hy}{a} + \underline{l}/c\left(\frac{3}{4}c + z\right)\right] \\
& + \cos 2\pi\left[-hy/a - \underline{l}/c\left(\frac{3}{4}c + z\right)\right] + \cos 2\pi\left[-h/a\left(\frac{a}{2} - z\right) - \underline{l}/c\left(\frac{c}{2} + z\right)\right] \\
& + \cos 2\pi\left[-h/a(a/2 - y) - \underline{l}/c\left(\frac{c}{4} + z\right)\right] + \cos 2\pi(-hx/a + \underline{l}z/c)
\end{aligned}$$

which simplifies to

$$\begin{aligned}
A = {} & 4\cos 2\pi\left[\frac{h + \underline{l}}{4} + \frac{\underline{l}z}{c}\right]\cos 2\pi\left[\frac{h + \underline{l}}{4} - \frac{hx}{a}\right] \\
& + \cos 2\pi\left[\frac{h + 2\underline{l}}{4} + \frac{\underline{l}z}{c}\right]\cos 2\pi\left[\frac{h - \underline{l}}{4} - \frac{hy}{a}\right] \quad \ldots\ldots\ldots(8)
\end{aligned}$$

Intensity of the X-ray Reflection

The calculation of the structure factor from a knowledge of the electron distribution in the unit cell has been derived above (3) but in practice it is the intensity of the diffracted beam which is observed and this must be related to the structure amplitude. From classical electromagnetic theory the amplitude of the wave scattered at a distance r from the source by a free electron is $\frac{A}{r}\frac{e^2}{mc^2}$ where A is the amplitude of the wave which set the electron vibrating and c is the velocity of light. In this expression the electric vector is assumed to be normal to the plane containing the incident and the scattered rays, but if it lies in this plane the amplitude becomes

$$\frac{A}{r}\frac{e^2}{mc^2}\cos 2\theta \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots(9)$$

2θ being the angle between the incident and the diffracted rays.

The ideal case for mathematical treatment is that of a parallel beam of monochromatic radiation falling on a perfect crystal. In actual fact crystals rarely fall into this category. They usually consist of a mosaic of small crystalline blocks which are inclined at small angles to each other with an irregular interruption of the intervening boundaries. The diffracted beam is thus produced on rotation through a small angle depending on the perfection of the crystal specimen. The absolute measurement of the energy of the diffracted beam is given by $\frac{E\omega}{I_0}$ where E is the energy measured during rotation ω and I_0 is the energy of the incident beam. Expressions (3) and (9) can be combined to give an absolute measurement of the structure amplitude from the equation

$$\frac{E\omega}{I_0} = \left[N\frac{e^2}{mc^2}F(hk\underline{l})\right]^2\lambda^3\,\frac{1+\cos^2 2\theta\,\delta v}{2\sin 2\theta} \quad \ldots\ldots\ldots\ldots(10)$$

for a crystal of very small volume δv of negligible absorption, λ being the wavelength employed and N the number of unit cells per unit volume of the crystal. $1 + \cos^2 2\theta$ is a factor employed to correct for the normal unpolarised state of the radiation.

Equation (10) gives the intensity-structure amplitude relation as applied to the majority of crystals.

For crystals of the perfect type the relation given by equation (11) must be used.

$$\frac{E\omega}{I_0} = \frac{8Ne^2}{3\pi mc^2}F(hk\underline{l})\lambda^2\,\frac{1+|\cos 2\theta|}{2\sin 2\theta} \quad \ldots\ldots\ldots\ldots\ldots(11)$$

The main difference between (10) and (11) is that $F(hk\underline{l})$ is squared in (10) whereas it only enters as a linear factor in (11).

When relative intensity measurements are required the expressions become much less complex

$$\frac{F_1}{F_2} = \sqrt{\frac{I_1P_1}{I_2P_2}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(12)$$

where I_1 and I_2 are the two intensities observed under the same conditions. F_1 and F_2 are the structure factors and P_1 and P_2 are the polarisation factors.

When the absorption of X-rays is appreciable in the crystal the intensities are corrected by a factor $e^{+\mu t}$ where t is the length of the mean path of the beam through the crystal and μ is the absorption coefficient for the wavelength employed.

If a crystal approximates to the mosaic type but with constituent blocks rather large for equation (10) to be valid there is a falling off of intensities of strong reflections. This effect is known as 'primary extinction' and may be reduced by quenching the crystal in liquid air to reduce the perfection of the specimen. 'Secondary extinction' is due to an artificial increase of the absorption coefficient for very strong reflections by the back-reflection of the diffracted beam from upper layers of the crystal.

Intensity measurements may be put on an absolute scale from first principles by means of equations (10) or (11) or as is more commonly done by comparison with a 'standard crystal' which has been calibrated directly.

Fourier Analysis

A quantity which is a periodic function of a single coordinate x can be represented by a one-dimensional Fourier series

$$F(x) = A_0 + A_1\cos(2\pi x/a + \alpha_1) + A_2\cos(4\pi x/a + \alpha_2) + A_3\cos(6\pi x/a + \alpha_3) + \ldots\ldots\ldots\ldots(13)$$

$$= \sum_{n=0}^{\infty} A_n\cos(2\pi n x/a + \alpha_n) \qquad \ldots\ldots\ldots\ldots(14)$$

where A is the amplitude and α the phase of each cosine term, and *a* is the distance within which the periodic function repeats itself. As has already been stated, in a crystal there exists a periodic variation in the electron density and this periodic function in three dimensions can be represented by a triple Fourier series

$$\rho(xyz) = \sum_{-\infty}^{+\infty} \sum_{-\infty}^{+\infty} \sum_{-\infty}^{+\infty} \left| F(hk\underline{l}) \right| \cos 2\pi (hx/a + ky/b + \underline{l}z/c + \alpha hk\underline{l}) \quad \ldots\ldots\ldots\ldots\ldots(15)$$

where $\rho(xyz)$ is the electron density at the point with coordinates (xyz) and $|F(hk\underline{l})|$ is the structure amplitude for the $(hk\underline{l})$ reflection as already defined by (3).

The electron density can be expressed as an expontential function

$$\rho(xyz) = \frac{1}{V_c} \sum_{-\infty}^{+\infty} \sum_{-\infty}^{+\infty} \sum_{-\infty}^{+\infty} F(hk\underline{l}) e^{-2\pi i (hx/a + ky/b + \underline{l}z/c)} \quad \ldots\ldots(16)$$

where Vc is the volume of the unit cell. $F(hk\underline{l})$ is a complex quantity involving both absolute value and the phase of the scattering factor, $F(hk\underline{l})$ and $F(\overline{hk\underline{l}})$ being conjugate .

If in a crystal possessing a centre of symmetry the origin of the coordinates (xyz) be taken at the centre of symmetry the phase angle $\alpha(hk\underline{l})$ which measures the displacement of the wave from the origin must be limited to values 0 or π and these two cases can then be dealt with by the assignment of appropriate signs to the coefficients $F(hk\underline{l})$. The series then becomes

$$\rho(xyz) = \frac{1}{V_c} \sum_{-\infty}^{+\infty} \sum_{-\infty}^{+\infty} \sum_{-\infty}^{+\infty} \pm F(hk\underline{l}) \cos 2\pi (hx/a + ky/b + \underline{l}z/c) \quad \ldots\ldots\ldots\ldots(17)$$

or for the two dimensional case

$$\rho(xy) = \frac{1}{A}\sum_{-\infty}^{+\infty}\sum_{-\infty}^{+\infty} \pm F(hk0)\cos 2\pi(hx/a + ky/b) \quad \ldots\ldots\ldots(18)$$

where A is the area of the projection.

In constructing the Fourier series appropriate to the structure under examination, the amplitudes are obtained from the X-ray data. Some other method must be used, however, in the determination of phase relationships. The most commonly employed is still that of setting up a trial structure and then finding how the structure factors for this compare with those obtained from the X-ray data. When a reasonable degree of fit has been obtained it is possible to proceed by Fourier analysis. A limited number of terms can be included in a Fourier series and from the resulting atomic positions the phase constants of a further series of terms can be fixed and a new summation set up. The method is thus one of successive approximation.

Direct information about the nature of the phase constants is possible if some of the atoms of high scattering power in the structure lie in special positions with respect to the symmetry elements. The contribution of those atoms to the structure factor for each plane may allow the phase constant of the whole structure factor to be decided.

The other direct method of analysis is that developed by Patterson.[19.] The form of Fourier series employed here represents a weighted average distribution of density about any point in the crystal. Since the coefficients of the terms employed are proportional to the squares of the structure factors no information as to the nature of the phase constants is necessary in setting up the series.

We have the result

$$\rho(xyz) = \sum_{-\infty}^{+\infty} \sum_{-\infty}^{+\infty} \sum_{-\infty}^{+\infty} |F(hk\underline{l})|^2 \cos 2\pi(hx/a + ky/b + \underline{l}z/c) \quad \ldots\ldots(19)$$

This function has maximum values at vector distances from the origin representing the separation between points of maximum electron density in the crystal.

Ambiguity arises in the interpretation of the data obtained from Patterson's analysis of a structure since it is only in the case of very simple structures that one can decide with certainty to which interatomic distances these vectors refer.

Experimental Methods

The recording of X-ray reflections is now almost invariably carried out by a photographic method involving the use of monochromatic radiation. The original Laue[2.] method, while affording information about crystal symmetry, introduces difficulties in the calibration of a 'white' X-radiation for intensity estimates. The Bragg[4.] ionisation spectrometer involving the individual measurement of reflections is laborious for the investigation of complicated structures. It does, however, give a value of absolute intensity and measurements on a few crystal planes by this method can be utilized to set intensities obtained by photographic methods on an absolute scale.

In single crystal investigations some modification of the Schiebold and Polanyi method is invariably used. Normally the number of reflections recorded is too great to permit of unambiguous indexing, since the rotation photograph only gives the value of $2\sin\theta$ and one index h, k, or l for each reflection. If the crystal is oscillated over a known small angular displacement then the ambiguities in assigning indices are considerably reduced. By moving film methods in which the angular position of the crystal at the time of reflection is given by a known film translation the difficulties of indexing accurately are overcome. The most commonly employed is that introduced by Weissenberg[14.] by which the reflections from individual layer lines of a rotation photograph are recorded on a film which moves in a direction perpendicular to the crystal axis in synchronisation with the crystal rotation.

The intensities of the reflections may be measured photometrically[22, 23.] or by means of accurate visual comparison. For this purpose the multiple film technique is useful[24.] since it is then possible to make a cross comparison of a known series of intensity scales.

The Fourier synthesis can be computed by numerical[25.] or combined mechanical and numerical methods.[26, 27.] The graphical representation of the structure is then obtained by preparing a contour map of electron density from the summation totals.

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THE CRYSTAL AND MOLECULAR STRUCTURE OF SOME ORGANIC POLYSULPHIDES

Ian MacPhail Dawson, B.Sc.

PART I

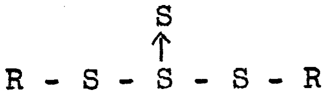
THE CRYSTAL AND MOLECULAR STRUCTURE OF SOME ORGANIC POLYSULPHIDES

Introduction

Although the organic polysulphides have been known for over fifty years the molecular structure has remained an unsolved problem. This has been due to the chemical instability of these compounds, the higher polysulphides readily decomposing to give a mixture of free sulphur and the mono- or disulphide. In previous structural investigations both parachor and viscosity measurements were utilized. From the former, Baroni[1] found support for the idea that the aliphatic tri-, tetra- and pentasulphides contain three sulphur atoms in linear arrangement with the additional sulphur atoms side-coordinated in the case of the tetra- and pentasulphides

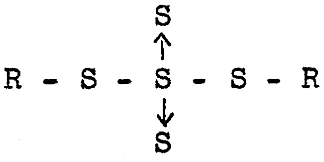
$R - S - S - S - R$

trisulphides



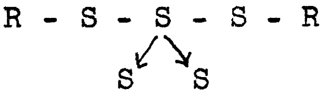
$$R - S - \overset{S}{\overset{\uparrow}{S}} - S - R$$

tetrasulphides



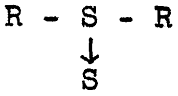
$$R - S - \underset{\underset{S}{\downarrow}}{\overset{\overset{S}{\uparrow}}{S}} - S - R$$

or



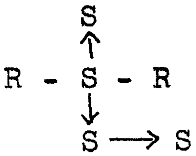
pentasulphides

Bezzi[2] on the other hand obtained viscosity measurements which indicated that in the mono-, di- and tetrasulphides with the same terminal alkyl groups the molecular length is constant. He concluded, therefore, that in the polysulphides we have a marked tendency to side-coordination and formulated the disulphides as

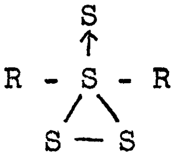


$$R - \underset{\substack{\downarrow\\ S}}{S} - R$$

and the tetrasulphides as



or

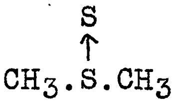


These measurements were carried out on liquid polysulphides of doubtful purity and consequently it is not surprising that the results are contradictory, and have been quoted with a considerable degree of reserve in the literature.

The only previous X-ray work in this field has been the unit cell determination of diphenyl disulphide by Egartner Halla and Schacherl[3.] Dimethyl disulphide has been studied by electron diffraction by Beach and Stevenson[4.] but in their work the difficulties of dealing with the molecule of this degree of complexity are well illustrated. Assuming the Pauling-Brockway C - S single bond radius of 1.81Å they calculate from an unresolved maximum at 1.94Å, representing the mean of the C - S and S - S interactions, that the S - S bond length is about 2.05Å. It was not found possible to distinguish directly between the structures I and II.

$CH_3.S - S.CH_3$

I.



$$CH_3.\overset{\substack{S\\ \uparrow}}{S}.CH_3$$

II.

The structure of sulphur itself has been studied in the rhombic form by Warren and Burwell[5.] by X-ray diffraction methods and in the gaseous phase by Chia-Si Lu and Donohue[6.] by electron diffraction methods. In both these investigations it was found that the S_8 molecule consists of an eight-membered ring. In rhombic sulphur Warren and Burwell postulate a puckered form of the ring as shown in Fig. I.

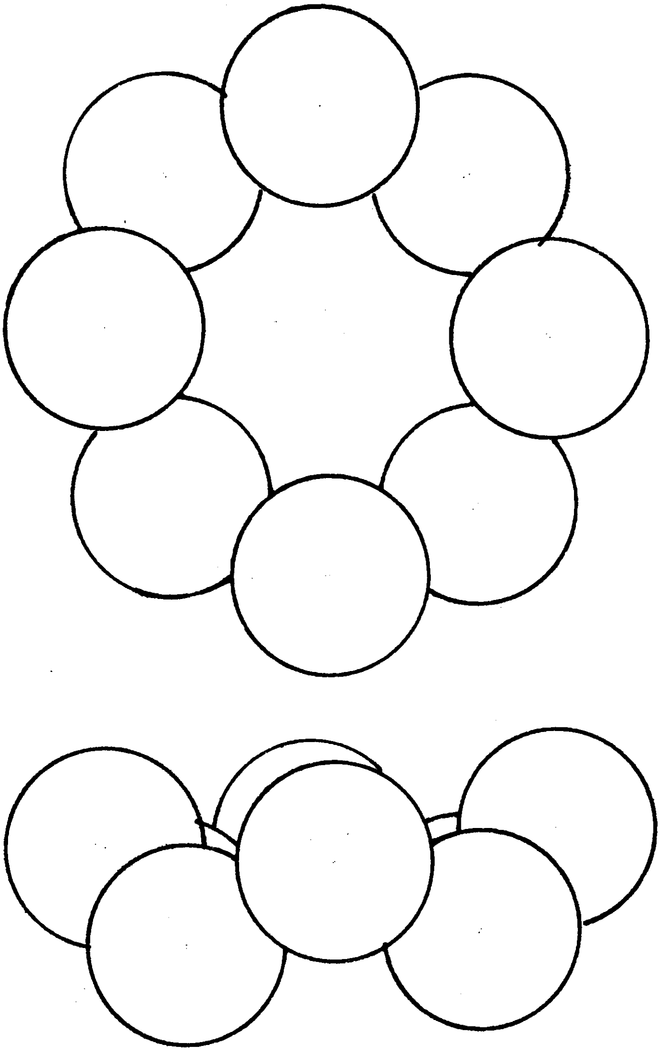
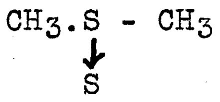


Fig. I

with an average S - S distance of 2.12Å ± 0.03Å and an S - S - S bond angle of 105.4°. For S_8 vapour Chia-Si Lu and Donohue have found that the radial distribution curve is compatible with the puckered ring form postulated by Warren and Burwell. The bond lengths and bond angles they found to be

S - S = 2.07 ± 0.02Å and S - S - S = 105° ± 2°

with rather a large amplitude of thermal vibration.

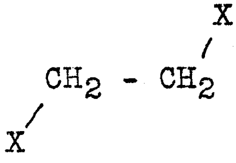
Stevenson and Beach in their work on dimethyl disulphide accept the Pauling-Huggins[7.] value of the single bond S - S distance as 2.08Å. Since the semi-polar distance, S → S in  $\mathrm{CH_3.\underset{\substack{\downarrow\\ S}}{S} - CH_3}$ should be about the double bond value of 1.90Å they conclude that the dimethyl disulphide molecule is a chain molecule and not the pyramidal form. This conclusion is further substantiated by the fact that the unresolved peak on the radial distribution curve representing the mean of the C - S and S - S interactions occurs at 1.94A. If the semi-polar S - S bond is 1.90A as Pauling and Huggins have deduced one would expect an interaction at about 1.86Å and not at the observed value of 1.94A.

This whole question of bond distances in connection with sulphur compounds is of some importance and will be dealt with more fully in the discussion at the end of this section. At this stage it will suffice to point out that the only direct measurements of the S - S covalent bond length have been obtained by Warren and Burwell[5.] on rhombic sulphur in which the value given was 2.12 ± 0.02Å and Zachariasen's[8.] value of 2.15Å obtained in a study of the inorganic trithionates. Since Zachariasen's work was completed in the early days of structure analysis and since Warren and Burwell's results are obtained from 48 relative intensity measurements which have not been put on a numerical basis it seemed desirable that in addition to a study of molecular configuration an effort should be made to obtain some rather more accurate data on the S - S bond radii and the S - S - S bond angle in this work.

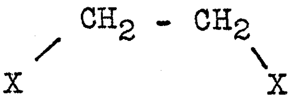
In connection with carbon-sulphur distances rather more information is available. Brockway and Jenkins[9.] in an electron diffraction study of dimethyl disulphide have determined the C - S bond length as 1.82 ± 0.03A. Lister and Sutton[10.] found a value of 1.90 ± 0.03Å for the C - S distance in dimethyl sulphone using the same technique. Toussant[11.] in an X-ray diffraction study of di p-bromophenyl sulphide reported a C - S distance of 1.75 ± 0.04Å and from a study of β-isoprene sulphone, Cox and Jeffrey[12.] report a length of 1.75Å for the C - S bond within the heterocyclic ring with a tetrahedral distribution of the four sulphur bonds.

Molecular configuration

The compound studied most fully in this investigation was β,β'-diiododiethyl trisulphide. From a study of ethylene chloride, ethylene chlorobromide and ethylene bromide by Beach and Turgevitch[13.] it was shown that these molecules exist in the vapour phase primarily in the trans form I.

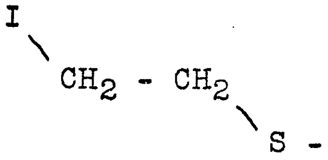


I.



II.

No evidence was found for the presence of a fraction in the cis form II. If φ be taken as the angle between the two halves of the molecule it was found that approximately 50% of the molecules at 300°K had a value of φ of 180° and that less than 5% had a value of φ below 90°. It thus seemed reasonable to assume as a first approximation that the β,β'-diiododiethyl trisulphide structure had a predominantly trans planar configuration of the groups in the molecule. Fortunately the structural determination was carried out by a method which did not in its ultimate stages pre-suppose any data regarding the configuration of the $I.CH_2.CH_2.S$ groups and in the discussion on page 17 the possibility of considerable departure from the trans planar orientation of this group in β,β'-diiododiethyl-trisulphide in the solid state will be examined more fully.



In the preliminary work on the structure of the organic polysulphides a survey of the unit cell and space group data was made for several compounds. This made it possible to select for more detailed examination those compounds, which, in the crystalline state, showed molecular symmetry. The possession of this symmetry limits the possible structural types, and simplifies the subsequent calculations. Of the four compounds examined, a two-fold axis of symmetry has been established in two and it has proved possible to carry the structure analysis to completion in the case of one of these, β,β' diiododiethyl trisulphide. The space group measurements and the preliminary data are given for the three compounds diphenyl-disulphide, di-p-tolyl-disulphide and β,β'-dichlorodiethyl-trisulphide followed by the detailed analysis of the β,β'-diiododiethyl-trisulphide structure.

RESULTS

Diphenyl-disulphide, $C_{12}H_{10}S_2$.

Crystal Data - M, 218.3; m.p. 59°; d, calc. 1.336, found 1.338; orthorhombic hemihedral, a = 8.11 ± 0.02, b = 23.67 ± 0.03, C = 5.61 ± 0.02A. Absent spectra, (h00) when h is odd, (0k0) when k is odd, (00l) when l is odd. Space group, D_2^4 - $P2_12_12_1$. Four molecules per unit cell. Molecular symmetry, none required. Volume of the unit cell, 1077A^3. Absorption coefficient for X-rays(λ = 1.54, A) = 40.7 per cm. Total number of electrons per unit cell = F(000) = 456.

Good crystals in the form of long needles or laths, with prominent development of the (110) faces are easily obtained by crystallisation from alcohol. Previous crystallographic studies are recorded by Groth[14.] and more recently an X-ray investigation has been made by Egartner, Halla and Schacherl[3.] whose determination of the unit cell and space group are in good agreement with the values reported above.

No clue to the molecular structure is provided by the space group data.

<u>Di-p-tolyl-disulphide $C_{14}H_{14}S_2$.</u>

<u>Crystal Data</u> - <u>M</u>, 246.4; m.p. 46°; <u>d</u>, calc. 1.240, found 1.246; monoclinic hemihedral, $a = 7.72 \pm 0.02$, $b = 5.76 \pm 0.02$; $c = 14.81 \pm 0.03A$, $\beta = 95.4^\circ \pm 0.2^\circ$. Absent spectra, (0k0) when k is odd. Space group, $C_2^2 - P2_1$ or $C_{2h}^2 - P2_1/m$. Two molecules per unit cell. Molecular symmetry, none required in $P2_1$, centre in $P2_1/m$. Volume of the unit cell = $656A^3$. Absorption coefficient for X-rays = 1.54A = 34.1 per cm. Total number of electrons per unit cell = F(000) = 260.

Well formed needle shaped crystals, with (001) predominantly developed, and (100) and (011) also present, are readily obtained from alcohol. A slight positive pyro-electric effect was noted, indicating the space group of lower symmetry, $P2_1$ as in the case of diphenyl-disulphide, neither cell dimensions nor symmetry provide any obvious clue to the molecular arrangement. A Patterson synthesis from the (h0$\underline{l}$) zone of reflections was prepared. In the summation 142 reflections were utilized.

The Patterson map obtained is shown in Fig. II. From this map it appears that the long axis of the molecules lie approximately parallel to the (010) plane and that their distance apart in projections along the b axis is about 4A. The complexity of the structure made it impossible to recognise peaks due to the interaction of bonded atoms.

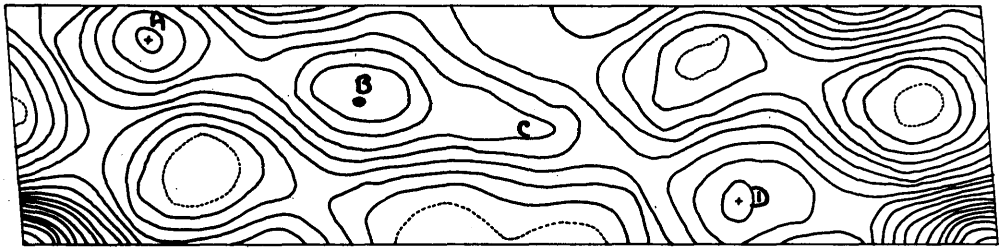
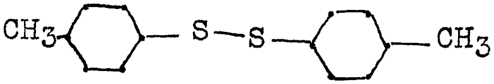


Fig. II . Patterson map (h0l) zone of di-p-tolyl-disulphide. Troughs in broken contour. Peaks marked A, B, C, D.

Several models of the trans structure



were utilized for trial and error calculations but the agreement obtained with this was never greater than 42%.

$\beta.\beta'$-dichlorodiethyl-trisulphide $C_4H_8S_3Cl_2$.

Crystal Data - *M*, 223.2 m.p. 27°; d calc. 1.491; found 1.494; orthorhombic, a = 5.47 ± 0.03, b = 8.01 ± 0.03, c = 22.5 ± 0.04A. Absent spectra, (h00) when *h* is odd, (00*l*) except when *l* = 4n. No data obtained for (0k*l*), (0k0) or (hk0). Space group, possibly $D_2^4 - P2_12_12_1$. Four molecules per unit cell. Molecular symmetry, none required from space group considerations but the quartering of (00*l*) indicates a two-fold axis. Volume of the unit cell, 998A³. Total number of electrons per unit cell = F(000) = 456.

Unfortunately this compound can only be crystallised with great difficulty and the high volatility of the few single crystals obtained do not permit of a complete analysis. The cell dimensions show some resemblance to those of the diiodo-compound, and the apparent quartering of the (00*l*) series of reflections is significant.

$\beta.\beta$

$\beta.\beta'$-diiodo-diethyl-trisulphide $C_4H_8S_3I_2$.

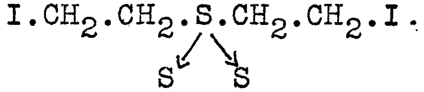
Crystal Data - *M*, 406.1; m.p. 110°; d, calc. 2.521, found 2.478; tetragonal trapezohedral, a = b = 6.01 ± 0.02, c = 29.40 ± 0.04A. Absent spectra (h00) when *h* is odd, (0k0) when k is odd, (00*l*) except when *l* = 4n. Space group $D_4^4 - P4_12_1$ (or $D_4^8 - P4_32_1$. Four molecules per unit cell. Molecular symmetry, two-fold axis perpendicular to (110). Volume of the unit cell, 1162A^3. Absorption coefficient for X-rays, $(\lambda = 1.54A) = 551.2$ per cm. Total number of electrons per unit cell = $F(000) = 744$.

Thin flat plates are readily obtained from alcohol-benzene mixtures, with (001) prominently developed. (011) and (101) are also displayed. A distinct tendency for cleavage parallel to (100) was noted.

For the β,β'-diiododiethyl trisulphide molecule the alternative structures possible are

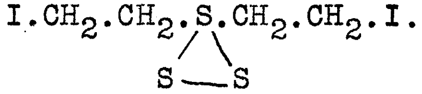
I. $\mathrm{I.CH_2.CH_2.S.S.S.CH_2.CH_2.I.}$

II.



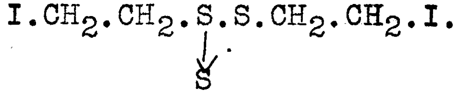
$$\mathrm{I.CH_2.CH_2.\underset{S\swarrow\ \searrow S}{S}.CH_2.CH_2.I.}$$

III.



$$\mathrm{I.CH_2.CH_2.\underset{S\!-\!S}{\overset{}{\underset{\diagup\ \diagdown}{S}}}.CH_2.CH_2.I.}$$

IV.



$$\mathrm{I.CH_2.CH_2.\underset{\underset{S}{\downarrow}}{S}.S.CH_2.CH_2.I.}$$

Structure IV is definitely excluded, since from the space group data it is seen that the molecule possesses an exact two-fold axis of symmetry.

The very long <u>c</u> axis and relatively small cross-section as well as the pronounced cleavage parallel to (100) suggests rod-shaped molecules arranged approximately parallel to the <u>c</u> axis. This is in favour of an extended zig-zag chain structure, although alternatives II and III are not definitely excluded.

In this structure the contribution of the iodine atoms to the

X-ray intensities is greatly in excess of that of all the other atoms combined. The distribution of scattering power in the molecule $C_4H_8S_3I_2$ from the sum of the atomic numbers, is 57% for iodine, 26% for sulphur and only 13% for carbon. The situation is clearly not such a favourable one for the application of the heavy atom method in resolving carbon positions as in the case of phthalocyanine[15.] or cholesterol iodide.[16.] The absorption coefficient for copper radiation of 551/cm. made considerable care necessary in the selection and cutting of crystal sections of uniform cross area for experimental work. Because of these factors the final Fourier projection obtained in this analysis (Fig.III) fails to give resolution of the carbon atoms although their positions may be inferred with reasonable certainty from the positions of other atoms.

Geometrical Structure Factors

The structure factors given by Lonsdale[17.] for space group $P4_12_1$ are evaluated for an origin taken at the intersection of one of the two-fold rotation axes of symmetry with a two-fold screw axis parallel to c. For projections along the c axis this will give an origin at an apparent centre of symmetry. In the present case, however, the only useful projection of the structure is obtained along the b axis and it is convenient to choose an origin at an apparent centre of symmetry in this projection. Such a point is obtained on a two-fold screw axis parallel to *b* and is conveniently chosen at $(-\frac{a}{4}, -\frac{b}{4})$ with respect to the tetragonal screw axis. This new origin is employed in the present analysis and it is situated at $(\frac{a}{4}, \frac{3b}{4}, \frac{c}{8})$ with respect to the origin adopted in the International Tables. The theoretical deduction of the modified structure facrors for space group $P4_12_1$ is given in the introductory section (p.16).

Analysis of Structure

Although an approach by the methods of Patterson analysis was possible in this structure the amount of evidence available from cell dimensions, cleavage, and an examination of the distribution of strong reflections in the ($h0l$) series facilitated the utilization of trial and error methods. Large structure factors were observed for the (008), (106), (10,14), (304) and (30,24) reflections and when traces of these planes are drawn on the *ac* projection it becomes possible to define the probable positions of the iodine atoms. On this basis various trial models were set up and atomic positions found which gave a satisfactory account of all the stronger reflections.

From this point the work proceeded by double Fourier series methods, utilizing the ($h0l$) structure factors. The projection first obtained gave clear resolution of the iodine positions but in it the sulphur coordinates were rather dubious. However, by means of the more accurate iodine coordinates obtained from the first projection it was possible to assign correct phases to a further series of reflections which had been omitted from the first summation because of phase ambiguities. The final series set up included 78 terms from a possible 135 up to the limit $2 \sin \theta = 1.6$. Films taken with exposures at 25ma./34KV did not show evidence of any of the missing reflections beyond this region; i.e. up to the limit of Cu radiation at $2 \sin \theta = 2.00$.

The results of the Fourier synthesis are given in Fig. I. This projection drawn on the (010) plane covers one complete unit cell, and includes four molecules. The line diagram (upper portion of Fig. I) indicates the molecular arrangement, and here probable positions for the carbon atoms are shown, although no direct evidence for these positions can be obtained from the electron density contours.

Coordinates and Dimensions

The projection of the structure shown in Fig.III. allows the coordinates of the iodine and sulphur atoms to be determined, the tetragonal symmetry making possible the direct measurement of all three coordinates of each atom. Table I lists coordinates for the atoms in the asymmetric crystal unit which is one half of the chemical molecule or one eighth of the total content of the unit cell. The coordinates of the remaining atoms follow from the symmetry operations of the space group $P4_1 2_1$ (p.17 of the theoretical introduction).

/Table I

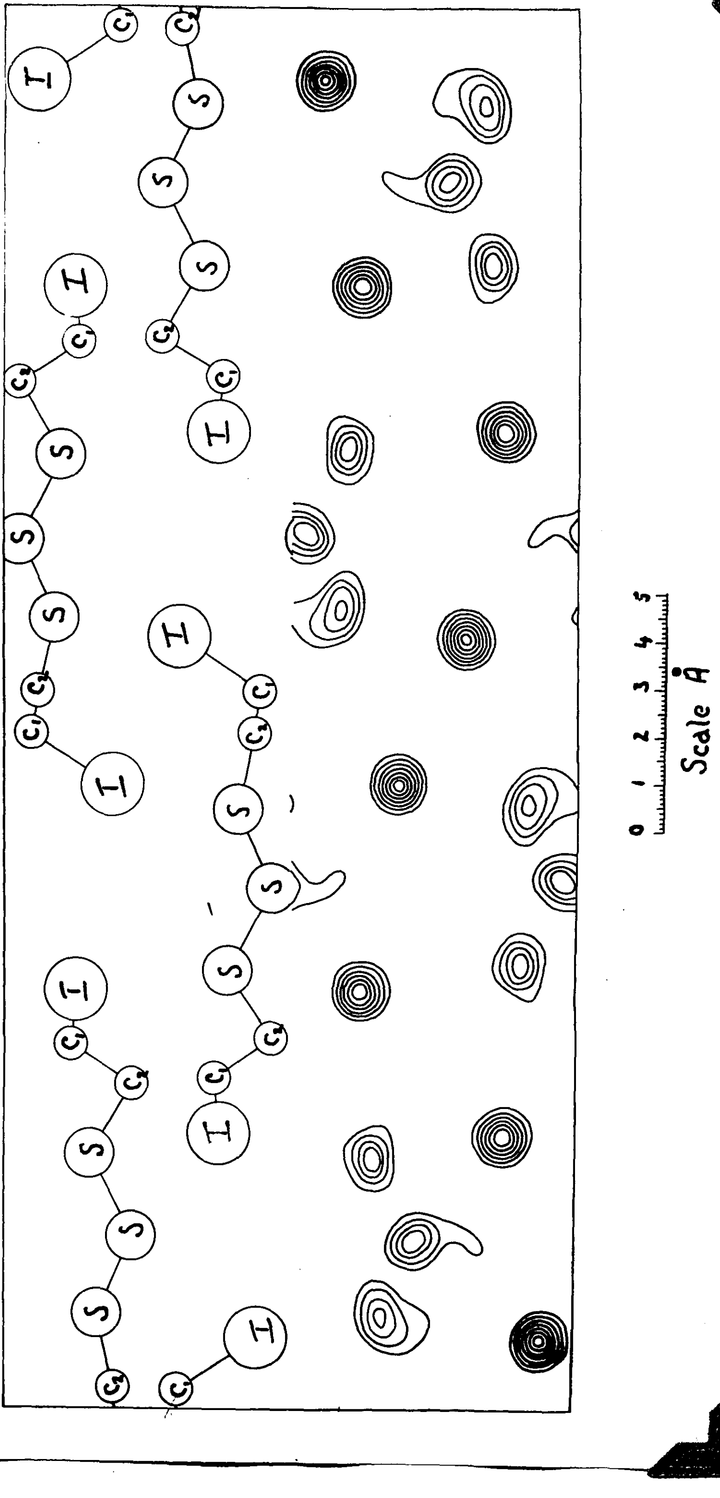


Fig. III. Projection along the b axis on to the (010) plane showing four molecules of β,β'-diiododiethyl trisulphide. Contour lines for the iodine atoms are drawn at density intervals of 10 electrons /A^2, and for sulphur at 4 electrons /A^2.

Table I

Coordinates with respect to tetragonal crystal axes. Origin on 2_1 axis parallel to b, at $(-\frac{a}{4}, -\frac{b}{4})$ from 4_1 axis.

	xA	$\frac{2\pi x}{a}$	yA	$\frac{2\pi y}{a}$	zA	$\frac{2\pi z}{c}$
I	-0.694	-41.7°	1.500	90°	-1.531	-18.7°
S_1	-1.96	-117.5°	1.81	108.5°	1.98	24.2
S_2	-2.68	-160.7°	2.68	160.7°	3.675	45°

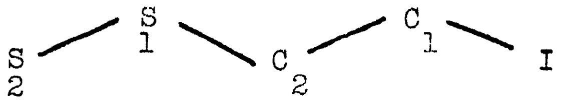
No clearly defined carbon peaks occur in the Fourier projection and in the calculation of the carbon coordinates the accepted single bond lengths and bond angles for the atoms in question were used (Table II).

Table II

Accepted values for bond lengths and valency angles

Parameter	rA	Parameter	A
C - C	1.54	∠CCS	112°[4.]
C - S	1.82[18.]	∠SSC	109°[4.]
C - I	2.10[18.]	∠CCI	109°[8.]

From these values we can deduce certain features of the structure. In particular, the measured distance between the sulphur atom S, and the iodine atom in the chain is only 3.74A. Assuming normal single bond values, a trans planar configuration for the group



This gives a clearance of 3.68A between carbon atom C_2 and the iodine atom on the neighbouring molecule at $-x, \frac{1}{2} + y, -z$.

On this basis the principal dimensions of the molecule are summarised in Table IV.

Table IV

Dimensions of the β,β'-diiododiethyl trisulphide molecule.

Bond Lengths	Bond Angles
S - S = 2.04 ± 0.04A	SSS = 113° ± 2°
C - S = 1.79A	SSC = 120°
C - C = 1.54A (assumed)	SCC = 96°
C - I = 2.05A	CCI = 100°

The shape of the molecule is interesting. It does, of course, possess an exact two-fold axis of symmetry in accordance with space group requirements. This axis is perpendicular to the (110) crystal plane and it passes through the central sulphur atom, S_2. Two views of the molecule are shown in Fig. II, representing projections along the symmetry axis and at right angles to the symmetry axis. The chain is almost fully extended but the atoms do not all lie in one plane. In fact, the plane of the central sulphur zig-zag is nearly at right angles to the plane of the carbon iodine zig-zag.

/Fig. IV

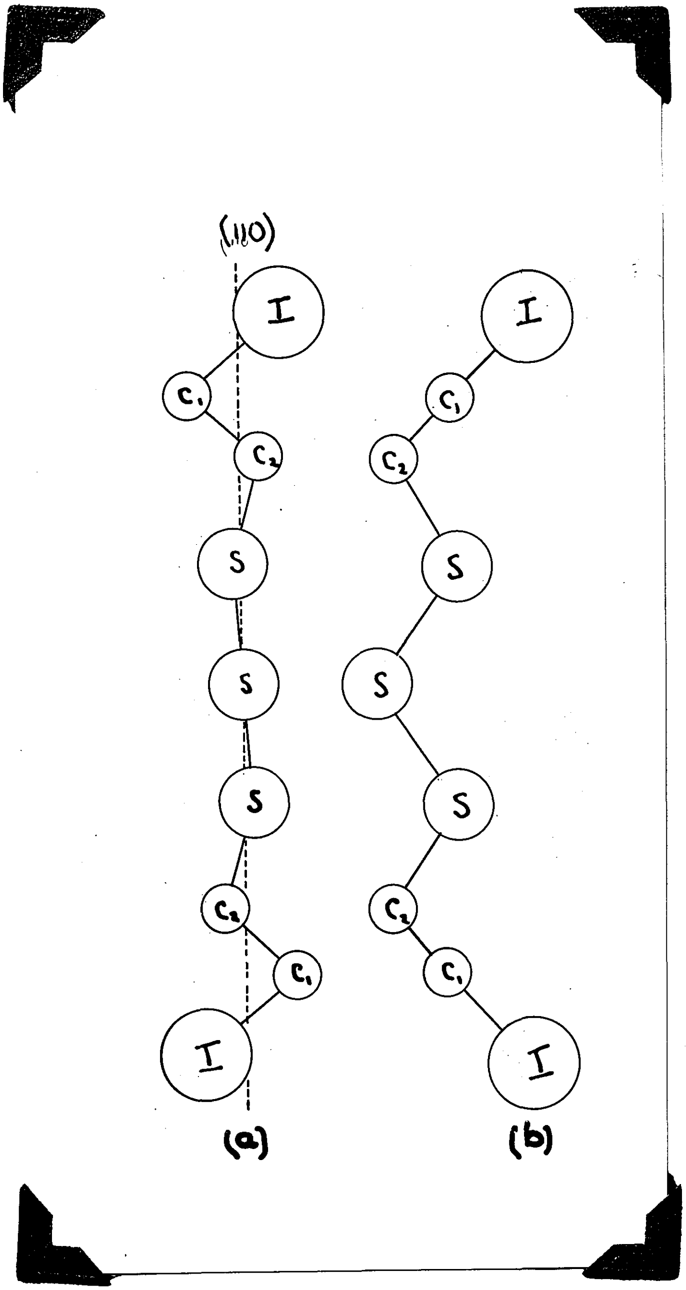


Fig. IV

a) Projection of the molecule along the axis of symmetry.

b) Projection of the molecules in a direction at right angles to that of (a).

Starting from a <u>trans</u> planar configuration, the present model may be derived by making a very small rotation about the S_1S_2 bond, a larger rotation about the C_2S_1 bond, and finally a rotation of 85° about the C_1C_2 bond.

Intermolecular Distances

The measured intermolecular distances are shown in Fig. V. The molecules are so packed that the iodine-iodine approach is never less than 4.44A. The shortest iodine-sulphur intermolecular distance is 3.80A, this being the distance from an iodine atom to a terminal sulphur atom of the S - S - S chain in the adjacent molecule. The distance from iodine to the central sulphur atom in the chain is 3.85A.

/Fig. V

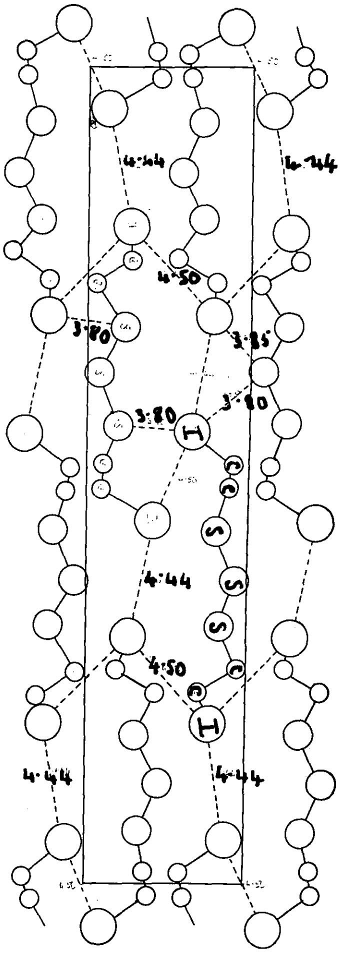


Fig. V.

Intermolecular distances in the β,β'-diiododiethyl trisulphide structure.

Discussion

The sulphur-sulphur bond length found is shorter than that reported for ortho-rhombic sulphur by Warren and Burwell,[5] or by the electron diffraction study of sulphur vapour.[6] These investigations favour the higher value of 2.12A. The value of 2.04A has, however, been reported for dimethyl disulphide.[4] We thus have both X-ray and electron diffraction evidence confirming a decrease in bond length of about 0.08A in the polysulphides as compared with sulphur itself.

In the present structure the S - S - S bond angle of about 113° is somewhat larger than the values previously reported which vary from 100° to 106°.

The intermolecular distances found are in good agreement with those previously reported for sulphur and iodine. The observed iodine van der Waals radius of 2.22A, when subtracted from the sulphur-iodine approach gives a van der Waals radius for sulphur of 1.58A. Warren and Burwell in their investigation of ortho-rhombic sulphur (loc.cit.) found a value of 1.65A. Both these values are less than the sulphur ionic radius of 1.85A but the analogy between ionic and van der Waals radii is not likely to be so good for divalent ions as for univalent ions (see Pauling,[18] p.174).

/Experimental

EXPERIMENTAL

Crystallization

Specimens of β,β'-diiododiethyl trisulphide were obtained from Mr. Kinnear of R.O.F., Porton. These were recrystallized from alcohol-benzene solution and yielded flat rectangular plates m.p. 110°.

Intensity Measurements

On account of high absorption ($\mu = 551$ per cm. for CuK) special precautions were necessary. It was found that the crystal specimens could be readily cut to uniform cross sectional area. That used for intensity measurements was in the form of a lath .13 mm. by .13 mm. and of length 0.65 mm. so that the necessity for absorption correction was reduced for the $(h0\underline{l})$ zone. The effect of the high absorption was further decreased by taking several films from crystals of slightly varying cross sectional area and making a comparison of the intensities measured Crystals were completely immersed in a beam of filtered copper radiation and the reflections recorded photographically by means of a moving film camera using the multiple film technique. The absolute scale of F values was not determined directly but was obtained by correlation with the calculated F values. The atomic scattering factor adopted was taken as a mean of the Thomas Fermi scattering factors[20.] and the Pauling Sherman[19.] scattering factors. An arbitrary temperature correction was applied by means of the

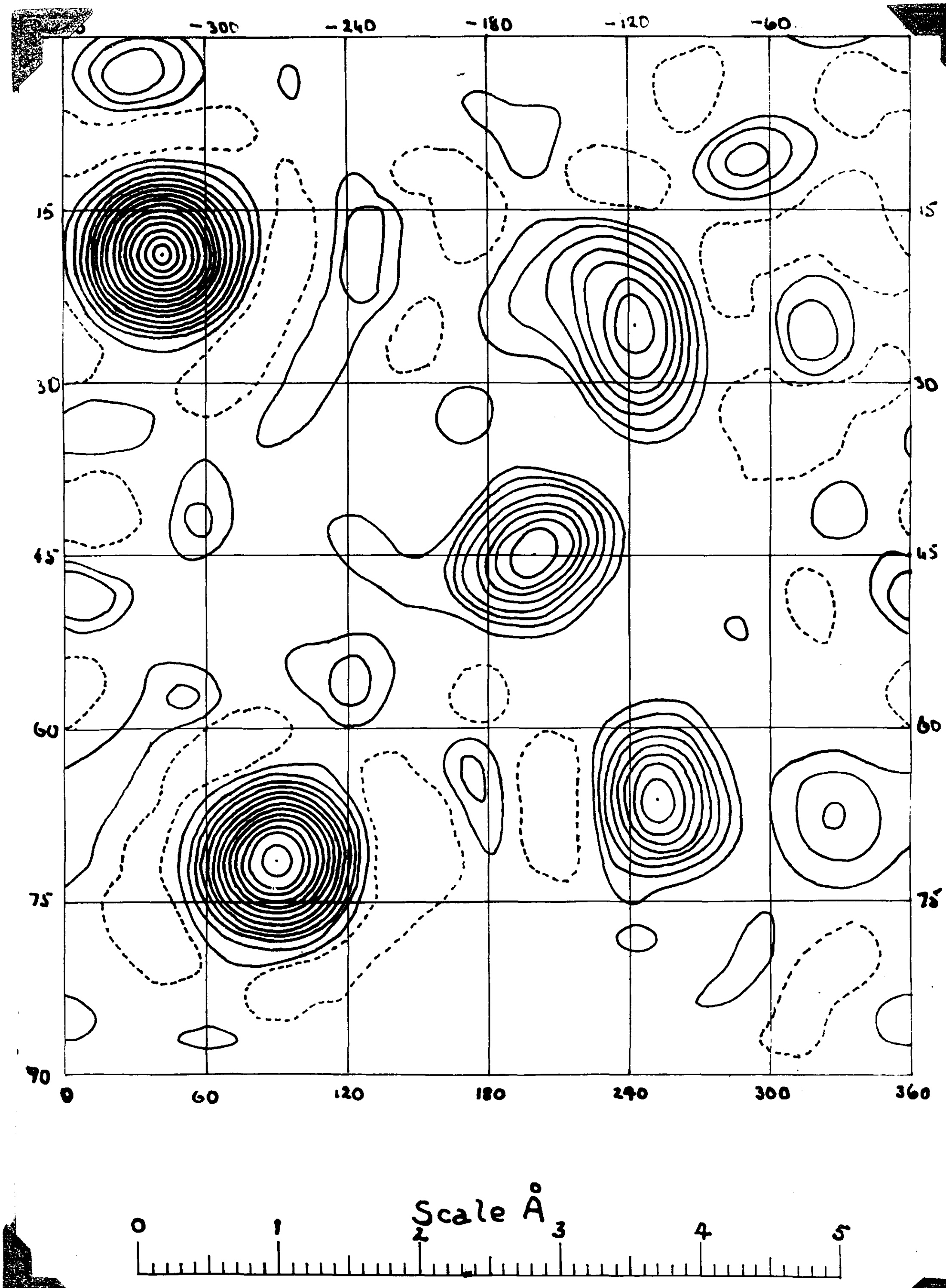
Debye Waller temperature factor using the value of Θ the characteristic temperature given for crystalline iodine. The sulphur contribution was then weighted $\frac{2}{7}$ on this scale and the carbon contribution was ignored in the evaluation of the calculated F's. This rather empirical treatment was found to be sufficiently accurate for the determination of the trial structure and in a final comparison of experimentally observed with calculated F's the degree of agreement was sufficiently good to preclude the necessity of drawing up an experimental scattering curve.

Fourier analysis

The electron density on the *a c* plane (010) was computed at 900 points on the asymmetric unit. The *a* axis was subdivided into 30 parts; the *c* axis into 120 parts, the intervals along *a* being 0.200Å and along *c* 0.245Å. The computation was effected by evaluating the series

$$\rho(xz) = \frac{1}{a\ c} \sum_{-\infty}^{+\infty} \sum_{-\infty}^{+\infty} F(h0\underline{l})\cos 2\pi(hx/a + \underline{l}z/c)$$

The summation was carried out with the aid of a mechanical sorting device.[21] This method gave the requisite 3° intervals along the *c* axis while still preserving the degree of accuracy of methods involving the use of three figure strips.[21] The positions of the contour lines were obtained by graphical interpolation from the summation totals by making sections of the rows and columns.



-300
-240
-180
-120
-60
15
30
45
60
75
90
15
30
45
60
75
0
60
120
180
240
300
360
Scale Å
0
1
2
3
4
5

The resulting contour map is shown in Fig. VI

Fig. VI

Coordinates assigned to atoms in the asymmetric unit. The zero electron line is shown dotted, each contour line representing an increase of 2 electrons per sq.A except for the iodine atoms where the increment is 5 electrons per sq.A. (The 2 electron line is omitted throughout.

/Diffraction effects

Diffraction effects

As seen from Fig. VI the diffraction effects in the Fourier projection are of an order sufficient to preclude any observation of the carbon positions. The method of removing these effects adopted in the study of Pt phthalocyanine[15.] has not been attempted because of the uncertain state of the S and C scattering curves in a compound of this type.

The coordinates of Table I and Fig. VI were used for a final calculation of the structure factors of all the (h0l) planes. The results are collected in Table V under "F calc." The agreement between observed and calculatedvalues of F, expressed in the usual way as the sum of all the discrepancies divided by the total measured structure factors, is 11.6% for the (h0l) reflections.

TABLE V

Measured and calculated values of the structure factor

hkl	sin θ λ= 1.54	F meas.	 calc.
004	0.105	33	+29
008	0.211	427	-424
0012	0.316	270	-275
0016	0.421	294	+282
0020	0.525	184	+172
0024	0.632	<56	-6
0028	0.738	139	-134

Table V continued

hkl	$\sin\theta$ $\lambda = 1.54$	F	
		meas.	calc.
101	0.130	81	+77
102	0.138	147	-124
103	0.150	81	+145
104	0.166	33	+64
105	0.184	81	+34
106	0.203	270	-275
107	0.224	107	-110
108	0.246	<33	+32
109	0.269	147	-150
1010	0.293	41	-28
1011	0.317	74	-96
1012	0.341	<41	-36
1013	0.365	57	-24
1014	0.390	229	+223
1015	0.415	<44	-30
1016	0.440	<45	-30
1017	0.466	<46	+36
1018	0.491	179	+156
1019	0.516	115	+132
1020	0.542	<48	+9
1021	0.568	117	+97
1022	0.594	156	-144

Table V continued

hkl	$\sin\theta$ $\lambda = 1.54$	F	
		meas.	calc.
1023	0.620	< 55	+12
1024	0.645	< 56	+34
1025	0.671	< 56	-32
1026	0.697	148	-149
1027	0.723	< 57	-45
1028	0.749	< 57	+10
1029	0.775	< 56	-43
1030	0.800	< 56	+39
200	0.256	205	-199
201	0.258	131	-89
202	0.261	197	+197
203	0.268	222	-165
204	0.277	82	-82
205	0.287	222	-181
206	0.301	82	-97
207	0.315	156	-141
208	0.331	220	+245
209	0.349	< 41	-52
2010	0.367	196	-200
2011	0.386	90	+97
2012	0.407	57	+33

Table V continued

hkl	$\sin\theta$ $\lambda = 1.54$	F	
		meas.	calc.
2013	0.427	155	+112
2014	0.449	< 44	+1
2015	0.471	164	+167
2016	0.493	< 45	-67
2017	0.516	70	+82
2018	0.539	131	+134
2019	0.562	< 49	-41
2020	0.586	107	-97
2021	0.610	73	-23
2022	0.634	82	+56
2023	0.658	90	-89
2024	0.682	< 55	+1
2025	0.707	91	-92
2026	0.731	91	-67
2027	0.756	82	-20
2028	0.781	< 56	+24
301	0.385	57	-46
302	0.387	41	+24
303	0.392	82	-63
304	0.398	270	-257
305	0.406	< 44	+31

Table V continued

hkl	sin θ λ= 1.54	F	
		meas.	calc.
306	0.415	< 44	-23
307	0.426	< 45	-16
308	0.438	156	-137
309	0.451	< 46	0
3010	0.465	46	+46
3011	0.481	123	+121
3012	0.497	171	+152
3013	0.514	74	+ 46
3014	0.532	74	-34
3015	0.551	< 50	0
3016	0.570	196	+191
3017	0.588	< 52	-25
3018	0.610	< 53	-3
3019	0.631	62	-67
3020	0.652	< 54	-39
3021	0.673	< 55	-35
3022	0.695	< 56	-42
3023	0.717	< 56	-41
3024	0.739	163	-145
3025	0.762	< 56	-17
3026	0.785	< 55	-47

/continued

Table V continued

hkl	sin θ λ= 1.54	F	
		meas.	calc.
400	0.512	< 47	+22
401	0.512	< 47	-24
402	0.514	246	-224
403	0.518	66	-30
404	0.523	< 48	-19
405	0.530	< 49	-59
406	0.536	115	+119
407	0.544	< 50	-26
408	0.551	< 50	+19
409	0.564	106	-26
4010	0.575	270	+230
4011	0.588	< 52	-34
4012	0.601	< 53	-21
4013	0.616	57	+85
4014	0.631	< 53	0
4015	0.647	< 54	+19
4016	0.663	< 54	+13
4017	0.680	< 55	-5
4018	0.689	180	-156
4019	0.716	< 56	+13
4020	0.734	< 56	-8
4021	0.753	< 56	-17

Table V continued

hkl	sin = 1.54	F	
		meas.	calc.
4022	0.773	<56	-90
4023	0.793	<55	-27
501	0.640	115	-117
502	0.642	<53	-25
503	0.645	<54	-11
504	0.649	98	+104
505	0.653	<54	-12
506	0.659	82	-60
507	0.666	82	+74
508	0.673	82	+69
509	0.682	107	+98
5010	0.692	58	+49
5011	0.702	82	-47
5012	0.714	<56	-64
5013	0.725	<56	+50
5014	0.738	56	+33
5015	0.752	57	-40
5016	0.766	90	-96
5017	0.781	74	-74
5018	0.799	<56	+19

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PART II

An X-ray Investigation of the Effect of Rickets on Bone Structure in Rats

Introduction

X-ray diffraction methods have been utilized to a considerable extent in the study of the structure of bone. Early work by de Jong[1.] showed that the diffraction pattern given by powdered bone closely resembled that given by the mineral fluorapatite and he concluded that there was a close similarity in the structure of the two substances. Taylor and Sheard[2.] and Roseberry, Hastings and Morse[3.] extended this comparison of the powdered bone diffraction pattern with the known mineral apatites and established that calcium carbonate and calcium phosphate are not present as such in the bone inorganic salt.

Clark[4.] first carried out an examination of intact bone sections and showed that in intact bone a certain degree of preferential orientation of the bone inorganic crystallites occurred.

This preliminary work was successful in establishing the apatite-like structure of the bone inorganic salt. There followed a period of intensive investigation of the exact composition of the inorganic material. Unfortunately this work has not yielded results capable of exact interpretation and considerable controversy still exists between the various workers in the field with regard to the final details in the structure.

In 1931 Hendricks, Hill, Jacobs and Henderson[5.] from chemical and X-ray diffraction data came to the conclusion that bone is essentially a carbonato- apatite or a carbonato hydroxyapatite $Ca_{10}CO_3(PO_4)_6H_2O$. Klement and Trommel[6] and Bredig[7] presented strong evidence in support of a hydroxyapatite rather than a carbonato- apatite structure for the bone inorganic salt. Bredig's work centred around a comparison study of the diffraction pattern given by the bone inorganic salt with that obtained from the natural and synthetic carbonato- and hydroxy- apatites. In 1936 Klement[8] claimed to have synthesised the bone inorganic salt by precipitating hydroxyapatite from solution in presence of carbonate. Gassman[9] disputed Klement's work and stated that it was not in agreement with the analytical results. He cited an experiment to show that no free calcium carbonate exists in bone. This point is still an open question.

In a study of the structure of tooth Bale, Le Fevre and Hodge[10] identified the main substance as hydroxyapatite with occluded or adsorbed carbonates. Subsequently Bale[11] showed that the unit cells of bone, dentine, hydroxyapatite and tricalcium phosphate are identical within the limits of experimental error. More recently Hendricks and Hill[12] have put forward a theory that the bone inorganic salt consists of a hydrated tricalcium phosphate type of compound instead of a hydroxyapatite and they maintain that sodium and carbonate are essential constituents of the compound for which they suggest an average composition $Ca_{8.50} \cdot Mg_{0.25} \cdot Na_{0.19} \cdot (PO_4)_{5.07} (CO_3)_{1.24}\ 2H_2O$. From an X-ray examination of powdered bone Dallemagne,[13] and

Dallemagne and Brasseur[14, 15.] suggest that the bone inorganic salt consists of a mixture of α tricalcium phosphate and calcium carbonate and that on ignition (900°C) a carbonato-apatite is formed.

In spite of the lack of detailed knowledge of the bone inorganic salt several studies of the X-ray diffraction patterns given by pathological bone have been made. In 1932 Shauman[16.] claimed that in rachitic bone the lines due to the organic matter appeared with much greater intensity than in ordinary bone. Clark and Mrgudich[17.] also reporting a study of rachitic bone claimed that the preferred orientation shown by the inorganic crystallites in normal bone is decreased and that there is an increase in the amount of amorphous material present. They concluded that there was a breakdown in the structure and organisation of the organic material of bone in rickets. Saupe[18.] reported the results of the effect of age, rickets, osteomyelitis and repair of broken bone on the bone diffraction pattern. Henschen[19, 20.] gave a detailed account of the changes occurring in the bone diffraction pattern with age and fatigue. Reed and Reed[21, 22.] in a study of the effect of rickets on the structure of bone claim that disorientation of the inorganic crystallites occurs in the rachitic diet and that reorientation takes place on healing.

In this investigation the primary aim was to compare the degree of orientation shown by rachitic bone diffraction patterns with that shown by normal bone. Work by Lamarque[23] has shown that considerable variations in the orientation shown by the inorganic crystallites in bone occur with variation in age or the location of the specimen selected for examination. The work was extended to include an examination of the orientation of the organic material present since preliminary measurements showed that the orientation of both bone constituents was inter-related.

Experimental Methods

All specimens were mounted on a non-rotating holder with the direction of the bone length perpendicular to the incident X-ray beam, and exposed to CuK_α radiation with a specimen to film distance of 4.01 cm.

1. Intact Specimens - Small portions of approximately 10 mm. by 0.2 mm. by 0.2 mm. were cut from central area of the femoral shaft. The location of the selected portion was standard throughout all the experiments.

2. Decalcified Specimens - Several methods are available for effecting decalcification but it is necessary to know first if the collagen of the bone remains unaltered in spite of the chemical treatment required. The diffraction pattern of fresh rat tail tendon was compared with the pattern obtained after it had been immersed in the decalcifying fluid for the time necessary to decalcify the small bone specimens. Neither dilute ammonium citrate (White's solution) nor 1% formic acid caused any change

in the orientation of the collagen pattern. Both methods were adopted in the routine decalcification of specimens. All such specimens were examined at room humidity.

3. Deproteinized specimens - The bone specimens were cut to dimensions suitable for X-ray examination, boiled in water for 24 hours and then subjected to trypsin digestion at pH 8 for 4 to 5 days. After thorough washing it was shown by Kjeldahl's method that the specimens contained no N; digestion was therefore complete.

4. Assessment of orientation - The 0002 reflection (Tables II and III) is the most intense oriented reflection occurring in the intact bone diffraction pattern. The angular measurement of this diffraction arc was taken as a measure of the degree of orientation shown by the inorganic crystallites.

Results

Experimental Material - The rats used in this investigation were divided into two groups according to dietary conditions, R group (18 animals) being fed a highly rachitogenic diet and S group (11 animals) being fed an adequate diet for control purposes. The R group on radiography showed marked rickets while all the animals in the S group on examination showed a normal type of bone formation.

X-ray data

The spacings measured, indices assigned and the orientation shown by the diffraction rings are tabulated for rachitic (R) specimens (Table I) and for control (S) specimens (Table II). The corresponding data for deproteinised specimens is given in Table III. The typical diffraction patterns obtained from rachitic and from deproteinised specimens are shown in Fig. 1. and Fig. 2. respectively. Table IV gives the spacings and orientation of the organic diffraction pattern a typical example of which is shown in Fig. 3.

Table I - Rachitic Bone (Intact specimens) from R group

Ring No.	d^* A$^{\circ}$	Indices	Orientation
1	12.8	Organic spacing	Equatorial
2	8.30	$01\bar{1}0$	Equatorial
3 & 4	5.4 to 4.2	Organic $01\bar{1}0$ $11\bar{2}0$	None
5	3.44	0002	Meridional
6 & 7	3.17	$01\bar{1}2$ $12\bar{3}0$	None
8 & 9	2.87	$12\bar{3}1$ $03\bar{3}0$	None
10	2.69	Organic spacing	Meridional

[*] All spacings correct to .05 A$^{\circ}$.

Table II - Normal Bone (Intact specimens from S group)

Ring No.	d* A°	Indices	Orientation
2	8.30	$01\bar{1}0$	Equatorial
3 & 4	5.3 to 4.2	Organic $01\bar{1}1$ $11\bar{2}0$	None
5	3.44	0002	Meridional
6 & 7	3.17	$01\bar{1}2$ $12\bar{3}0$	None
8 & 9	2.87	$12\bar{3}1$ $03\bar{3}0$	None
10	absent		

* All spacings correct to .05 A°.

Table III - Deproteinized bone specimens

Ring No.	d A°	Indices	Orientation
2	8.14	$01\bar{1}0$	Equatorial
3	5.28	$01\bar{1}1$	None
4	4.22	$11\bar{2}0$	Equatorial
5	3.45	0002	Meridional
6	3.31	$01\bar{1}2$	None
7	3.22	$12\bar{3}0$	Equatorial
8	2.96	$12\bar{3}1$	None
9	2.86	$03\bar{3}0$	Equatorial
10	2.72	$02\bar{2}2$	None
11	2.55	$03\bar{3}1$	None

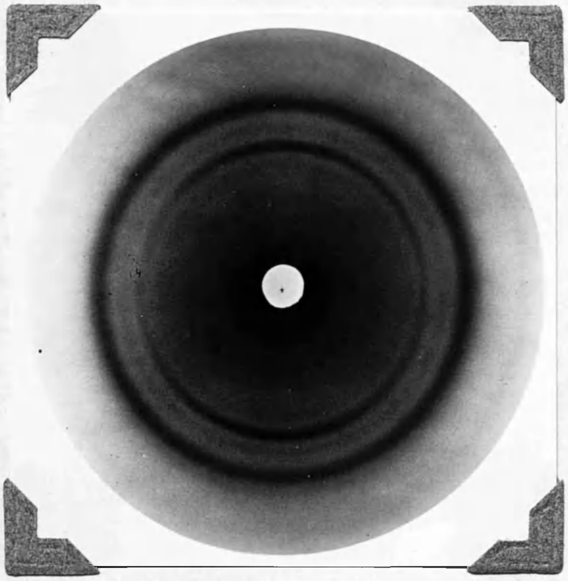

Fig. 1. Rachitic bone diffraction pattern

Fig. 2. Deproteinised bone diffraction pattern

Table IV - Decalcified bone (rachitic, R, and control, S, groups)

Spacing A^{o}	Orientation
11.2 - 13.8	Equatorial
4.1 - 5.5	Slight Equatorial
2.8 - 2.9	Meridional

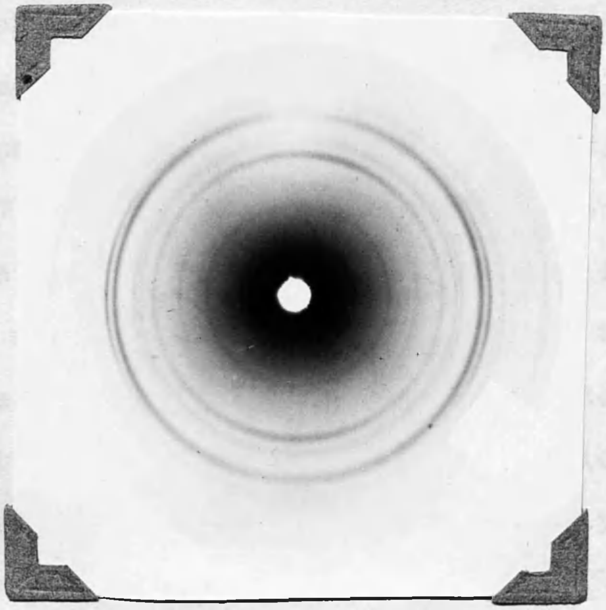

Fig. 3. Decalcified bone diffraction pattern

1. The inorganic spacings have the same value within the limits of experimental error in control (S) group and in rachitic specimens (R group).

2. The organic spacings 1 and 10 occur in rachitic specimens only. The broad diffraction ring from 5.4 - 4.2 A^{o} in the rachitic specimens is reduced to 5.3 - 4.2 A^{o} in the control specimens. This ring has been shown to be composed of an organic spacing and the inorganic $01\bar{1}0$ and $11\bar{2}0$ superimposed. Its weaker intensity in the control specimens is due to a decrease in the scatter from the organic constituent; the inorganic spacings remain the same in intensity in the rachitic and control whereas organic spacings 1 and 10 do not appear in the control.

From Table IV (deproteinised specimens) it is seen that on removal of protein there is a considerable sharpening of the inorganic diffraction pattern, without, however, any appreciable change in the spacings measured. The composite diffraction rings 3 and 4, 6 and 7, and 8 and 9 in the intact specimen are resolved in the deproteinised specimens. The decrease in intensity of the diffraction rings with decrease in spacing is much more marked in the case of intact specimens. For deproteinised specimens there are two further unoriented diffraction rings 10 and 11 appearing whereas with intact rachitic bone only the oriented spacing 10 occurs (Tables II and IV).

The organic spacings measured from the decalcified specimens (Table V) are very similar to those given by Astbury (1940) for collagen and gelatin for which structures have been proposed by Astbury and Bell (1940).

In both rachitic and normal bone there is uniaxial orientation of both organic and inorganic material in a direction parallel to the bone length. This is most clearly indicated, in the case of the inorganic material, by the most intense oriented 0002 spacing (Table II and III). The orientation is most pronounced at the centre of the shaft and decreases towards the epiphysial area where there is no detectable orientation. At the centre of the shaft there is no significant difference in the orientation shown by the rachitic and normal specimens.

Discussion

As has already been stated in the introduction (p.3), considerable uncertainty still exists as to the exact composition and structure of the bone inorganic salt. The large number of diverse theories current in this field are almost certainly due to the fact that intact bone gives a diffraction pattern rather lacking in fine detail (Fig. 2. p. 9). Indeed, the lack of detail is such that the features discernible can be identified qualitatively with almost all the apatite-like numerals (2) (3). In these circumstances it is natural that a considerable degree of speculation around the available data should take place. In an effort to increase the definition shown by the diffraction pattern Klement and Trommel[6.] have carried out calcination at

900°C. This treatment is, however, open to objection in that, in addition to removing the organic matter present, a considerable degree of dehydration of the inorganic material takes place, so that the final structure can have very little resemblance to the natural material. Dallemagne and Brasseur[13, 14, 15.] have relied on treatment with 6% potassium glyceride to remove protein from intact bone and report that the resultant diffraction pattern is very similar to that given by intact bone. This finding was taken to show that deproteinisation by this method produced no structural changes in the inorganic salt. This was a reasonable assumption at the time of experiment. In a search for a mild form of deproteinisation it was decided in this investigation that enzymic digestion provided considerable advantages over chemical treatment, since the degree of specificity involved would better protect the inorganic salt from attack and in addition the hydrogen ion concentration for such a method is much nearer the value pertaining in normal tissue fluids. The result obtained was of great interest in that a considerable increase in the definition of the diffraction pattern was observed. The spacings measured and the intensities recorded remained the same; within the limits of measurement in both types of photograph.

Now a sharpening of this kind in the diffraction pattern given by a crystalline substance would indicate (in the absence of chemical changes involving a subsequent recrystallisation) that the micro-crystals had increased in size. The situation is analogous to that existing between mosaic and perfect crystals discussed in the theoretical introduction (p.   ). The crystal type has changed from extremely small mosaic blocks giving a diffuse diffraction pattern and large blocks giving a much sharper pattern. The conclusion must be that on deproteinisation by trypsin forces causing distortion of the inorganic micro-crystals are released, and the volume over which the structure is regular is increased. The forces involved are most probably those of attraction between protein and the surface areas of the inorganic crystallites since it has been found that the organic and inorganic material of bone has an orientation in the bone which is always closely similar in degree and direction. It would be of little advantage, however, to speculate further on the exact nature of the forces between the collagenous protein of bone and the inorganic material until more is known of the structures involved. Since the method of deproteinisation utilised in this investigation has yielded rather more data than previous methods, more exact information of the structure of the inorganic constituent should now be possible.

Comparison of results obtained from normal and rachitic specimens of rat femora

Mention has already been made of the results obtained by Clark and Mrgudich[17.] and by Reed and Reed[21, 22.] in the introductory section. In both investigations a disorientation of the inorganic material in rachitic bones is reported but only in the former paper is there any reference to the organic constituent.

A careful evaluation of orientation was made by the method described in the experimental section and preliminary experiments with our material confirmed the following findings:- 1. the orientation of the inorganic crystallites varies with the location on the bone of the specimen selected for examination (Reed and Reed 1942), and 2. the degree of orientation shown by the specimen varies considerably with age (Cagliotì and Gigante 1936). It was established that these two factors have a similar influence on the organic constituent. The disturbing effects of these factors were eliminated so far as possible from our experiments by selecting specimens from the same location on each bone examined and by making comparisons between animals of the same age.

No significant difference in orientation of either inorganic or organic material was found between normal and rachitic specimens. This result is at variance with that reported by Clark and Mrgudich (1934) and by Reed and Reed (1942 and 1945). This difference cannot be ascribed to differences in technique since the X-ray methods used by us and by Reed and Reed are identical (personal communication). Analysis of Reed and Reed's published data shows that there is a significant difference between the mean ages of the animals whose bones showed poor orientation and good orientation; the older bones showed the better orientation. It is possible that the change in orientation reported by these workers is a natural change occurring with age rather than a response to anti-rachitic treatment. The fact that no orientation is found could be explained if the portion of the shaft used for diffraction studies had not been affected by the rachitic process. This is, however, difficult to reconcile with the very low ash content (circa 36%) of these bones as compared with the bones of the well fed animals (circa 60%).

This series of experiments has thus failed to give support to the idea of a peripheral action of vitamin D in the sense suggested by Reed and Reed.[21.]

The findings can be summed up most simply by stating that the deterioration in mechanical strength observed in rachitic bones is not associated with any chemical or structural alteration in the bone material. The strength of the bone material seems to depend entirely on the relative proportions of organic and inorganic material. This serves to emphasize the conclusion arrived at earlier by Bell and Cuthbertson (1943) that the organism has arrive at a well defined and constant method of laying down bone. Neither calcium deficiency, nor hormone treatment, nor the rachitic disturbance seem able to disarrange this pattern so far as our methods are able to reveal it.

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